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学 位 論 文 要 旨

Production and characterization of lignocellulolytic enzymes from a white-rot fungus *Porodaedalea pini*

白色腐朽菌マツノカタワタケ由来のリグノセルロース分解酵素の生産および特性解明

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White-rot fungi have been received much consideration for their valuable enzyme systems which can effectively degrade lignocellulose biomass. Although white-rot fungi are commonly found on hardwood species, *Porodaedalea pini* commonly attacks softwood species, such as *Picea, Pinus, Larix, Tsuga,* and *Pseudostuga*. Compared with studies on lignocellulolytic activities of the hardwood-degrading fungi, very limited information is available about the lignocellulolytic activities of *P. pini* to date. The objectives of the present study are to characterize the lignocellulolytic enzymes secreted by *P. pini.* The relationships were discussed between enzyme production and wood chemical component changes during degradation of *P. jezoensis* wood by *P. pini*. In addition, the secreted fungal proteins were identified by proteomic analysis. Furthermore, the major glycoside hydrolase, β-glucosidase produced by this fungus was partially purified and biochemically characterized, being evaluated as a novel enzyme for potential use in biotechnological applications.

The degradation of *Picea jezoensis* wood by *P. pini* was examined under laboratory conditions. Changes in wood chemical components of *P. jezoensis* and lignocellulolytic enzymes secreted by the fungus were investigated over various periods. The enzyme secretion and mass loss of *P. jezoensis* wood were significantly increased after 60 days of degradation. After that, they gradually increased with increase in incubation time. The wood degradation resulted in changes of wood chemical composition: total lignin, holocellulose, and α-cellulose contents were decreased, while extract contents (hot water and 1% NaOH) were significantly increased. The pH of degraded wood was significantly lower than that of sound wood. During degradation process, *P. pini* secreted xylanase, β-glucosidase, and endoglucanase with higher

activities than those of exoglucanase and cellobiose dehydrogenase. On the other hand, this fungus secreted manganese(II)-dependent peroxidase at the highest level in ligninolytic enzymes, followed by lignin peroxidase and/or versatile peroxidase and laccase. These results indicate that *P. pini* degrades all wood chemical components of *P. jezoensis* and secretes a variety of lignocellulolytic enzymes, and these enzymes might synergistically act on wood chemical components degradation.

The time-course changes in glycoside hydrolase activities and profiles of the proteins secreted by *P. pini* were investigated in modified Norkran's medium with microcrystal cellulose as a carbon source. Based on the enzyme assay results, the activity of β-glucosidase was the highest among the glycoside hydrolases produced by the fungus. The β-glucosidase activity increased with increase in cultivation time and attained to the highest level at 28^{th} day. Two dimensional electrophoresis pattern of the extracellular proteins was similar among different time cultivation with a little difference in spot number. The number of separated protein spots was 415, 370, 375, 410, 446, and 426 in average for 8, 16, 20, 24, 28, and 32 days of cultivation, respectively. Twenty seven protein spots were analyzed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and identified by peptide mass fingerprinting. The proteins were identified as those involved in the carbohydrate transport and metabolism (4 proteins), amino acid transport and metabolism (7 proteins), aromatic compound metabolism and oxidative stress responses (2 proteins), cellular processes and signaling (5 proteins), and hypothetical proteins (9 proteins), based on their biochemical roles. In addition, one protein spot with theoretical and observation molecular mass about 70 kDa and pI 6.02 was homologous to the glycosyl hydrolase family 1. The major glycoside hydrolase, β-glucosidase produced by *P. pini* in Mandels medium with microcrystal cellulose as a carbon source was partially purified and biochemically characterized. Among glycoside hydrolases produced by *P. pini*, the β-glucosidase showed the highest activity. β-Glucosidase was partially purified 7.43 fold with a specific activity of 2.85 μkat/mg protein by employing ion exchange and hydroxyapatite chromatographies. The optimum temperature for the enzyme fraction activity was 70°C, and the enzyme fraction could retain the stability after pre-incubation at 60°C for 30 min. The enzyme fraction had optimum pH value 4.0, and it was stable at pH in the range from 3.0 to 6.0 after pre-incubation for 30 min. The presence of Cu^{2+}, Na^{+}, Ni^{2+}, and DMSO inhibited the activity of the β-glucosidase fraction, whereas K^{+}, Ca^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, β-mercaptoethanol, and SDS did not influence the β-glucosidase fraction activity. The *P. pini* β-glucosidase fraction was not glucose tolerant, while this enzyme fraction was not influenced by ethanol up to 20% concentration. The β-glucosidase fraction from *P. pini* had high substrate affinity for *p*NPG, supported by the K_m value 0.289 mM.

In conclusion, the white-rot fungus *P. pini* is considered to be a novel source for glycoside hydrolases production, especially β-glucosidase. The results obtained in this study provide some important evidences to understand the characteristics of *P. pini* during wood degradation and lignocellulolytic enzyme production. Based on the results, it is considered that *P. pini* β-glucosidase can be used to contribute to lignocellulosic bioethanol production.